The Effect of the Rediae of *Cryptocotyle lingua* (Creplin, 1825) (Digenea : Heterophyidae) and *Himasthla leptosoma* (Creplin, 1829) (Digenea : Echinostomatidae) on the Glycogen and Free Sugar Levels of the Digestive Gland and Gonad of *Littorina littorea* (Linnaeus, 1758) (Gastropoda : Prosobranchia)

BY

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(4 Text figures)

INTRODUCTION

Histological and histochemical work (Cheng & Snyder, 1962; James, 1965; James & Bowers, 1967a, 1967b; Cheng, 1967) has suggested that digenean germinal sacs bring about the degradation of glycogen, present in the cells of the host's digestive gland and gonad, to glucose which is absorbed and immediately resynthesized to glycogen or catabolized. Support for these, *in vivo*, histochemical studies comes from the demonstration by McDaniel & Dixon (1967) that the redia of *Cryptocotyle lingua* readily absorbs exogenous glucose which is then synthesized into endogenous disaccharides, trehalose and polysaccharides.

In contrast, the *in vitro* studies of Friedl (1961a, b,), Cheng, (1963) and Vernberg & Hunter (1963) suggest that germinal sacs are unable to digest exogenous glycogen or utilise exogenous sugars.

In this investigation, quantitative comparisons of the glycogen and quantitative and qualitative comparisons of the free sugars present in the digestive gland and gonad are made on parasitized and non-parasitized male and female *Littorina littorea* (Linnaeus, 1758). The glycogen and free sugar levels of the rediae of *Himasthla leptosoma* and *Cryptocotyle lingua* are also described. The main objectives of this investigation were to determine the number and source of the parasites' free sugars and to determine the effect of redial infection on the free sugars and glycogen of the molluscan host.

MATERIALS and METHODS

The host, *Littorina littorea,* was collected from Tŵr Gwylanod, Aberystwyth, mid-west Wales in January and February of 1970 and 1971. The molluscs were maintained, overnight, in an aquarium tank at 12° C and dissected the following day. The following tissues were dissected from the host at a room temperature of 20 - 21° C:

1. The digestive gland and gonad of non-parasitized male *Littorina littorea.*
2. The digestive gland and gonad of non-parasitized female *Littorina littorea.*
3. The digestive gland of *Littorina littorea* parasitized by the rediae of *Cryptocotyle lingua* or *Himasthla leptosoma,* excluding the parasites (the gonad was invariably completely destroyed).
4. The rediae of *Cryptocotyle lingua.*
5. The rediae of *Himasthla leptosoma.*

The tissues were washed in artificial sea water (Barnes, 1965). Excess moisture was removed by means of a current of cold air from a laboratory blower and the fresh

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weights were recorded. The glycogen and free sugar content of the tissues was determined using the following procedures:

a) **Glycogen**

The determinations were carried out on two sets of tissues (1 - 5) obtained from *Littorina littorea*. Glycogen was extracted from the tissues as follows:

1. 10 volumes of 70% ethanol were added to the tissue which was extracted at 5° C for 18 hours. Centrifugation was carried out to obtain a clear supernatant which was decanted and discarded.
2. The tissue was re-suspended in a further 10 volumes of 70% ethanol and homogenized with a Teflon-glass Potter-Elvejhem homogenizer. Centrifugation was carried out to obtain a clear supernatant which was again decanted and discarded.
3. The residue was washed in 4 volumes of diethyl ether and 70% ethanol (3:1), centrifuged and the supernatant decanted and discarded.
4. Step 3 was repeated and the supernatant decanted and discarded.
5. The residue was re-suspended in 10 volumes of 10% trichloroacetic acid and heated to 80° C. On cooling, centrifugation was carried out to obtain a clear supernatant which was decanted and stored for glycogen determination.
6. The residue was re-suspended in 10 volumes of ice-cold 10% T.C.A. and extracted at 3° C for 30 minutes. Centrifugation was carried out to obtain a clear supernatant which was decanted and added to the supernatant from step 5.
7. The combined supernatants from steps 5 and 6 were mixed with 30% potassium hydroxide (3:17) and boiled at 100° C for 20 minutes. On cooling, 1.2 volumes of 95% ethanol were added. The solution was mixed, boiled, cooled and centrifuged. The supernatant was decanted and the precipitate was quantitatively analysed for glycogen.

The glycogen was assayed by the method of Montgomery (1957) using a Unicam SP 500 spectrophotometer at a wavelength of 490 nm.

b) **Free sugars**

Each tissue was homogenized in a volume of 10% isopropanol sufficient to make the final tissue concentration 200 mg/ml. The homogenates were centrifuged to obtain clear supernatants. Each supernatant was stored at –60° C for 12 hours, prior to chromatographic analysis and re-centrifuged immediately before spotting on to a chromatogram. Preliminary experiments indicated that satisfactory separation and recovery of sugars could be achieved without prior recourse to deproteinisation and desalination of the supernatant.

Thin-layer chromatography was carried out on Eastman Chromagram Sheets (Type 6064, cellulose without fluorescent indicator, supplied by Kodak Ltd., Kirkby, Liverpool), in the Eastman Developing Apparatus at a room temperature of 20 - 21° C. Twelve μl of the test solution were applied to the chromagram in 0.5 μl aliquots. One way runs using the solvent n.propanol: water (2:1) were carried out. The sheets were developed to a distance of 18 cm in the solvent, dried in a current of cold air and redeveloped in the same direction, in the same solvent, to the same distance. Standard marker mixtures of sugars at known concentration were run alongside the test solutions to facilitate identification of the sugars present in the test solutions. Detection of the separated sugar spots was achieved by dipping the dried chromagrams in the Anilene reagent of Smith (1960). The chromagram sheets were then heated in a pre-set electric oven at 110° C for 3 minutes. Pentoses gave red-brown colours and the other sugars gave yellow-brown colours on a pale brown background.

The weights of the free sugars present in the test solutions ($\mu g/g \times 10^{-3}$ tissue) were estimated using the method of Purdy & Truter (1962). The analyses were repeated using an original volume of 16 μl of test solution and the weights of the free sugars present were again determined. The mean weight of the sugars present in each tissue was determined from these results.

The whole procedure was repeated on a second sample of winkles and the mean weight of sugar ($\mu g/g \times 10^{-3}$ tissue) was again determined. The difference in the results never exceeded $\pm$ 13%, indicating the repeatability of the technique.

RESULTS

a) **Glycogen**

The quantity of glycogen in the non-parasitized digestive gland and gonad of male and female *Littorina littorea* is similar (Figure 1) but is lower in the parasitized tissue and lower still in the rediae of *Himasthla leptosoma* and *Cryptocotyle lingua*.

b) **Free sugars**

There is little qualitative difference in the free sugar in the tissues (Table 1). Glucose, maltose and ribose are common to all of the tissues but lactose is found only in the non-parasitized male.

The quantitative estimation of the free sugars (Figures 2 to 4) show that there is little difference in the amount

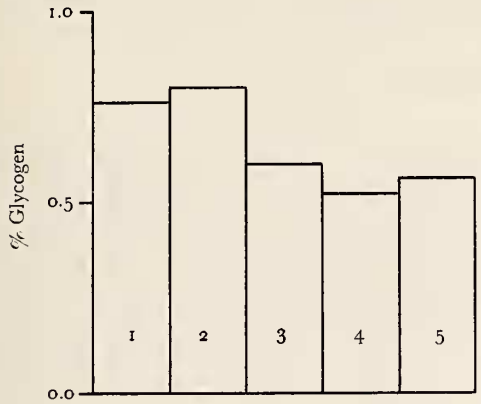

Figure 1

The glycogen in the digestive gland and gonad of *Littorina littorea* and in the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma* expressed as a mean percentage of the fresh tissue weight.
Key to tissue numbers:
Tissue

1. The digestive gland and gonad of non-parasitized male *Littorina littorea*
2. The digestive gland and gonad of non-parasitized female *Littorina littorea*
3. The digestive gland of *Littorina littorea* infected by the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma* but excluding the parasites
4. The rediae of *Cryptocotyle lingua*
5. The rediae of *Himasthla leptosoma*

of glucose and ribose present in non-parasitized male and female tissues, but the male contains 30% more free maltose than the female. The parasitized tissue contains 25% more free ribose, over 100% more free glucose and 25% more free maltose than the non-parasitized male tissue.

The amount of ribose in the rediae of *Himasthla leptosoma* and *Cryptocotyle lingua* (Figure 2) is higher but the free glucose is 100% lower in *H. leptosoma* and 50%

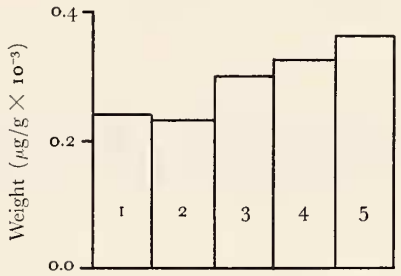

Figure 2

The mean weight of ribose (μg per g × 10^{-3} of tissue fresh weight) present in the digestive gland and gonad of *Littorina littorea* and in the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma*. The tissues (1-5) are listed in Figure 1 and in the text

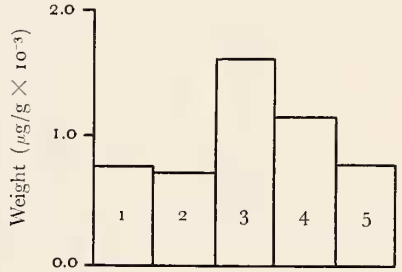

Figure 3

The mean weight of glucose (μg per g × 10^{-3} of tissue fresh weight) present in the digestive gland and gonad of *Littorina littorea* and in the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma*. The tissues (1-5) are listed in Figure 1 and in the text

Table 1

The Free Sugars Present in the Digestive Gland and Gonad of *Littorina littorea* and in the Rediae of *Cryptocoytle lingua* and *Himasthla leptosoma*.

	Tissue Number				
Free Sugars	1	2	3	4	5
Ribose	++	++	++	++	++
Glucose	++	++	++	++	++
Maltose	++	++	++	++	+
Lactose	+	a	a	a	a

++ = sugar present in measurable amount.
+ = sugar present in trace amount.
a = sugar not detected.
The tissues (1-5) are listed in Fig. 1 and in the text.

lower in *C. lingua* (Figure 3) than in the parasitized tissue. The amount of free maltose in the parasitized tissue is similar to that in *C. lingua* but maltose was detected only as a trace in *H. leptosoma* (Figure 4).

The non-parasitized tissue has between 30 and 50% less free ribose than the rediae (Figure 2) but the free glucose is similar to that in *Himasthla leptosoma* (Figure 3) and approximately 50% lower than in *Cryptocotyle*

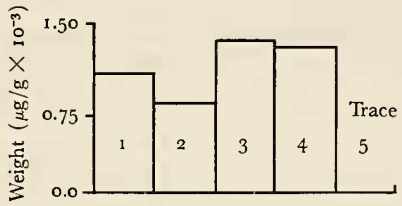

Figure 4

The mean weight of maltose (μg per g × 10^{-3} of tissue fresh weight) present in the digestive gland and gonad of *Littorina littorea* and in the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma* The tissues (1-5) are listed in Figure 1 and in the text

lingua (Figure 3). The free maltose in *C. lingua* is 19% higher than in non-parasitized male tissue and 56% higher than in non-parasitized female tissue.

DISCUSSION

The fact that the non-parasitized male and female tissues contain more glycogen than the infected tissue suggests that a breakdown of host glycogen is occurring in the parasitized tissue either as a result of enzymatic action by the parasites or as a result of the release of digestive enzymes from host cells damaged by the feeding processes of the rediae (McDaniel & Dixon, 1967).

Further evidence for the breakdown of glycogen in the parasitized tissue comes from the demonstration of the presence of both maltose and glucose in the parasitized digestive gland at much higher levels than those found in the corresponding non-parasitized tissues. Thus, these results appear to vindicate the histochemical work referred to earlier.

The fact that maltose and glucose were the only products of glycogen digestion detected in the parasitized tissues may indicate that the breakdown of this polysaccharide is by hydrolytic rather than phosphorylytic enzyme activity.

The presence of maltose, glucose and glycogen at lower levels in the rediae than in the parasitized hepatopancreas may suggest that free sugars taken up from the host are mostly catabolized rather than re-synthesized into stored glycogen.

The metabolic rate of the rediae of *Himasthla leptosoma* is significantly higher than that of the rediae of *Cryptocotyle lingua* (Thomas, 1971) and it seems probable, therefore, that the lower concentrations of free maltose and glucose in *H. leptosoma* than in *C. lingua* may be attributable to their being catabolized at a higher rate.

The presence of ribose in relatively high concentrations in the rediae indicates that it has a role to play in their metabolism, in spite of the suggestion by Vernberg & Hunter (1963) that rediae do not utilize ribose.

SUMMARY

The glycogen and free sugar in the hepatopancreas of non-parasitized male and female *Littorina littorea* are similar but infection by the rediae of *Cryptocotyle lingua* and *Himasthla leptosoma* results in a depletion in host glycogen accompanied by an increase in free maltose and free glucose. This suggests that host glycogen is broken down as a result of host or parasitic hydrolytic enzyme activity. The products of digestion of host glycogen are absorbed by the rediae and mostly catabolized but some may be re-synthesized.

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